of protein in a prolonged period. The composite scaffold could induce the adhesion and osteogenic differentiation of MSCs.
http://dx.doi.org/10.1016/j.jot.2016.06.106

181
LiCl PROMOTES CHONDROGENIC DIFFERENTIATION OF BMSCs IN INFLAMMATORY CONDITIONS INDUCED BY IL-1 THROUGH SUPPRESSING NF-κB SIGNAL PATHWAY

Peng Cheng, Kun Chen, Xingli Du, Fengjing Guo, Anmin Chen
Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, China

Introduction: It is a complex process to regulate bone marrow mesenchymal stem cells' (BMSCs) chondrogenic differentiation, especially in inflammatory conditions. This study aimed to investigate the effect of LiCl in chondrogenic differentiation of BMSCs in inflammatory conditions and the possible mechanism in this process.
Subjects and Methods: BMSCs were treated with IL-1 in the process of chondrogenic differentiation. Along with IL-1, one group was treated with 10 nM LiCl and the other group with 10 nM GSK-3β. For each group, the glycosaminoglycan (GAG) amount was quantitatively tested and the mRNA of Sox 9, Collagen 2a, and Aggrecan were tested by RT-qPCR. The total NF-κB protein, p-NF-κB protein, and the NF-κB protein in cytosol or nucleus were tested by Western blot.
Results: Our results demonstrated that the index of chondrogenesis, such as the mRNA of Sox 9, Collagen 2a, Aggrecan, and the amount of GAG, were significantly decreased in the IL-1 group. However, in inflammatory conditions induced by IL-1, the indexes of chondrogenesis in LiCl group were significantly increased. Additionally, total NF-κB protein was increased, p-NF-κB protein was significantly decreased, and NF-κB protein in the nucleus was significantly decreased. All of these in GSK-3β group were just opposite to the LiCl group.
Discussion and Conclusion: These results strongly suggest that the ability of BMSCs' chondrogenic differentiation were decreased in inflammatory conditions induced by IL-1, but LiCl could enhance the ability although with IL-1. The possible mechanism was LiCl promotes the phosphorylation of the NF-κB protein and the transfer into nucleus, and then suppresses the NF-κB signalling pathway.
http://dx.doi.org/10.1016/j.jot.2016.06.107

191
DECELLULARISATION OF THE PORCINE TENDON-BONE INTERFACE FOR TISSUE ENGINEERING

Kai Xu [a,b], Lara Kuntz [b], Katharina Kümpel [b], Peter Föhr [b], Alexandra Wagner [b], Jutta Tübel [b], Rüdiger v. EisenhartRothe [b], Rainer H. Burgkart [b]
[a]*Department of Orthopedics, Tongji Hospital, Huazhong University of Science and Technology (HUST), Wuhan, China*
[b]*Clinic of Orthopedics and Sport Orthopedics, Klinikum rechts der Isar, Technical University of Munich (TUM), Munich, Germany*

Background: High stress levels occur at interfaces between mechanically dissimilar materials. Therefore, fixation of soft tissue to bone, e.g. after bone tumour removal, has a high incidence of failure after surgical repair. Regenerating tendon/ligament-to-bone insertions, "entheses", by tissue engineering offers a promising solution to this challenge. Decellularisation of porcine Achilles' tendon entheses might elicit physiologically relevant scaffolds for tissue engineering. In this study, we establish a protocol to decellularise porcine entheses as potential scaffolds for interface tissue engineering.
Subjects and Methods: Achilles' tendons with attached calcaneus were harvested from six month old pigs and cut into 2x6x10mm^3 samples. Group 1 was chemically treated with a PBS solution containing 0.5% sodium dodecyl sulfate (SDS) and 1% triton100 (2s2t) for 48 hours, group 2 for 72 hours. In both groups, incubation was performed on a shaker at room temperature with exchange of detergents every 24 hours. A custom-made hydrostatic decellularisation device was used for group 3. Untreated/ PBS-treated samples were used as controls. Decellularisation was assessed histologically with HE staining and Masson staining. Cell counts were performed on randomised regions of interest (ROI = 200x200μm^2) in tendon, bone, and interface and subsequently averaged. According to the DNeasy Blood & Tissue protocol, DNA content was assayed after the decellularisation process. Biomechanical system (zwick i1120, Zwick/Roell, Germany; sensor type: KAF-Z, 2.0mV/V = 2.5KN, A.S.T.GmbH Dresden, Germany) was applied to test samples' mechanical characteristics, such as rupture load, stiffness, and Young's modulus. Statistical analysis was conducted using GraphPad Prism 6 software. Treatment groups were compared to evaluate decellularisation efficiency.
Discussion and Conclusion: Treatment of porcine Achilles' tendon entheses with 0.5% SDS + 1% Triton washing for 72 hours resulted in the most efficient and complete decellularisation. Structure and integrity of the enthesis matrix was preserved. Following decellularisation treatment of 2S2T 72 hours, there was a 98% reduction in local cells ($p<0.01$). While, all treated groups exhibited a statistically significant reduction in DNA versus the untreated group ($p<0.01$), no statistically significant difference was observed among all the treated groups of DNA remained radio. The biomechanical tests indicated that, samples throughout the process had similar mechanical characteristic of rupture loads, stiffness, and Young's modulus to the untreated group. In the future, the decellularized scaffolds will be recellularised with mesenchymal stem cells to investigate matrix effects on differentiation of cells as well as developing scaffolds for enthesis tissue engineering.
http://dx.doi.org/10.1016/j.jot.2016.06.108

273
SCREENING KEY FACTOR FROM SENSORY NERVES REGULATING ON MACROPHAGE IN PROMOTING BONE DEFECT HEALING AND ITS MECHANISM

Junqin Li
Xijing Hospital, China

To investigate the mechanism of bone defect healing is of great significance for taking corresponding measures to promote the bone defect healing. Macrophages, as one of the essential factors in the process of bone defect healing, can promote bone formation and inhibit bone absorption. However, the factors regulating macrophages during bone repair is unknown. Nerves can manipulate the balance of the differentiation of bone cells and macrophages and can also promote bone repair. In the previous study, it was found that sensory nerves can promote bone defect healing significantly, while motor nerves did not have an evident role. In this study, we used iTRAQ to compare the differential expression proteins in sensory and motor nerves and anticipate obtaining the functional neuropeptide regulating macrophages. We found 19 factors' expression in sensory nerves significantly higher than in the sciatic nerve. By using a literature review, we focused on MIF (macrophagemigration inhibitory factor), which can affect migration and phagocytosis of macrophages and is also closely related to bone repair. We further used a rat tibia drilling model as a subject to detect the distribution of MIF during bone repair. We found MIF abundantly distributed in the lysosomes of macrophages, showing macrophages englobe MIF; the macrophages comprising MIF were distributed in the bone repair active region, such as the bone remodelling region around the trabecular and blood vessels. The cells are abundant in the primary callus stage than the mature callus stage. These results indicate that MIF is involved in the regulation of macrophages in promoting bone defect healing, and the regulation may be through endocytosis of macrophages.
http://dx.doi.org/10.1016/j.jot.2016.06.109

281
AMECM/DCB SCAFFOLD PROMPTS SUCCESSFUL TOTAL MENISCUS REGENERATION IN A RABBIT TOTAL MENISCECTOMY MODEL

Zhiguo Yuan
The PLA General Hospital, China

Objective: The meniscus lacks self-repair ability because of its unique structural features, so the treatment of meniscus lesions is a big challenge all the time. The aim of this study is to construct a composite meniscus scaffold (AMECM/DCB scaffold) with an acellular meniscus extracellular matrix (AMECM) and decalcified cancellous bone (DCB), test the physicochemical characteristics *in vitro* and immunological properties *in vivo*, implant the scaffolds in New Zealand rabbits that underwent total meniscectomy, and evaluate the meniscus regeneration and articular cartilage protective effect.
Methods: We utilised an acellular meniscus extracellular matrix (AMECM) and demineralized cancellous bone (DCB) to construct three different kinds of three-dimensional porous meniscus scaffolds (AMECM scaffold, DCB scaffold, and AMECM/ DCB scaffold). We detected the physicochemical characteristics of the three different scaffolds, including micro-structure analysis through SEM (scanning electron microscope), the scaffold composition detection through histological and biochemical analysis, and mechanical property testing with BOSE mechanical testing machine. We implanted the three different scaffolds subcutaneously in the rats to assess the immunological rejection of the scaffolds. We then seeded the meniscus fibrochondrocytes into the scaffolds and then observed the microstructure with SEM, tested the cytotoxicity of scaffolds through live/dead cell staining, and detected the glycosaminoglycan (GAG) and collagen content secreted by the fibrochondrocytes in the three scaffolds after 3, 7, and 14 days. We implanted the three different scaffolds into New Zealand rabbits which underwent total meniscectomy and then evaluated the meniscus regeneration and cartilage protective effect of the three different groups through macroscopic observation, histological analysis, X-ray, MRI, biomechanics tests, and RT-PCR at three and six months.
Results: The SEM results showed that all the three different scaffolds possessed a three-dimensional porous structure and good porosity. The GAG content of AMECM/DCB scaffold and AMECM scaffold was higher than that of the DCB scaffold. The biomechanical property of AMECM/DCB scaffold was superior to those of the